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ii) Running Head: $p\text{CO}_2$ accelerates biofouling succession

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Abstract

Rising carbon dioxide concentrations are rapidly altering the carbonate chemistry of the oceans and this phenomenon, termed ocean acidification, may have far-reaching consequences for marine community and ecosystem dynamics. Such impacts remain poorly understood due to the difficulty of manipulating $p\text{CO}_2$ at the ecosystem level to mimic realistic fluctuations that occur on a number of different timescales. It is unclear how robust ecosystems are to intermediate-scale $p\text{CO}_2$ change; how quickly can communities at various stages of development respond to acidification, and, if high $p\text{CO}_2$ is relieved mid-succession, do these effects persist, are the effects reversed by alleviation of $p\text{CO}_2$ stress, or are the effects worsened by departures from prior high $p\text{CO}_2$ conditions to which organisms had acclimatized. As nearshore CO_2 often fluctuates throughout succession, a further unresolved issue is the relative importance of direct acidification effects on primary colonization vs. indirect effects. Here, we used reciprocal transplant experiments along a shallow water volcanic $p\text{CO}_2$ gradient to assess the importance of the timing and duration of high $p\text{CO}_2$ exposure (i.e. discrete events at different stages of successional development vs. continuous exposure) on patterns of colonization and succession in a benthic fouling community. We monitored community development both before and after reciprocal transplantation of communities at eight weeks until the end of the experiment at twelve weeks. We show that succession at the acidified site was initially delayed (less community change by eight weeks) but then accelerated over the next four weeks. These changes in succession led to homogenization of communities maintained in or transplanted to acidified conditions, and altered community structure in ways that reflected both short- and longer-term acidification history. These community shifts are likely a result of interspecific variability in response to increased $p\text{CO}_2$ and changes in species interactions. High $p\text{CO}_2$ altered biofilm development, allowing serpulids to do best at the acidified site by the end of the experiment,

although early (pre-transplant), negative effects of $p\text{CO}_2$ on recruitment of these worms was still detectable. The ascidians *Diplosoma* sp. and *Botryllus* sp. settled later and were more tolerant to acidification. Overall, transient and persistent acidification-driven changes in the biofouling community, via both current and post exposure, could have important implications for ecosystem function and food web dynamics.

Introduction

Anthropogenic carbon dioxide enrichment of the atmosphere and subsequent decreases in pH in the ocean are well documented (Feely *et al.*, 2004; Tans, 2009). The rate of change of ocean pH is unprecedented in recent geological history (Hönisch *et al.*, 2012) and the biological implications of these rapid chemical changes are being realized across a wide range of taxa (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). In coastal marine ecosystems, changes in seawater $p\text{CO}_2$ are occurring on a number of different timescales: diurnally with photosynthesis and respiration, days to weeks with the lunar cycle and upwelling dynamics, seasonally due to both biotic and abiotic forcing, and over many decades with anthropogenic forcing (Hofmann *et al.*, 2011; Boyd *et al.*, 2016; Henson *et al.*, 2017). It is clear that incorporating natural fluctuations in $p\text{CO}_2$ is necessary for making better predictions of the biological response to ocean acidification (Shaw *et al.*, 2013; Small *et al.*, 2015; Boyd *et al.*, 2016). Although a few studies have addressed this point at diurnal timescales (e.g. Clark & Gobler, 2016; Li *et al.*, 2016), it is unclear how marine ecosystems respond to longer term (weeks to months) $p\text{CO}_2$ fluctuations and whether these effects can be reversed (transient vs. persistent effects) if $p\text{CO}_2$ stress is relieved (but see Vaz-Pinto *et al.*, 2013).

In communities influenced by disturbance (i.e., most communities), the effects of fluctuating $p\text{CO}_2$ will be mediated by direct effects of acidification on settlement and recruitment and by indirect effects mediated by the interactions between early and later successional species. Ocean acidification can influence settlement of planktonic stages and recruitment into benthic populations (Cigliano *et al.* 2010; Brown *et al.*, 2016), and resident organisms influence subsequent settlement (i.e. secondary colonization, Osman *et al.*, 1989). However, the importance of colonization history in shaping community structure and succession in light of $p\text{CO}_2$ or pH heterogeneity at a variety of temporal scales has yet to be addressed. One challenge to disentangling effects of acidification on the succession and development of marine communities is that, to date, our understanding of the biological effects of ocean acidification is primarily informed by studies of single species in isolation. Such studies show how ocean acidification might influence organisms through changes in energetic demand (Garilli *et al.*, 2015; Harvey *et al.*, 2016), reproduction and development (Ross *et al.*, 2011), growth rate (Kroeker *et al.*, 2013a), development of defensive structures (Sanford *et al.*, 2014), and behaviour (Milazzo *et al.*, 2016). However, it is not easy to extrapolate from single-species studies to assess the effects of ocean acidification on community development, structure, and function. To anticipate ecosystem-level changes, it is essential to understand responses of multi-species assemblages to acidification. Early studies exposed pre-settled communities to acidification in laboratory conditions (e.g. Hale *et al.*, 2011), but deeper understanding of recruitment and settlement processes requires *in-situ* $p\text{CO}_2$ manipulation (e.g. Brown *et al.*, 2016) or observation.

Shallow water CO_2 seeps allow the study of intact communities and have been increasingly used as natural laboratories, providing insights into the community- and ecosystem-level effects of

acidification. Changes in community composition, structure, and losses in diversity have been documented along natural $p\text{CO}_2$ gradients for both macro-algal (Kroeker *et al.*, 2011; Porzio *et al.*, 2011; Johnson *et al.*, 2012; Connell *et al.*, 2013; Baggini *et al.*, 2014, 2015; Linares *et al.*, 2015) and macro-invertebrate (Hall-Spencer *et al.*, 2008; Cigliano *et al.*, 2010; Fabricius *et al.*, 2011, 2014; Donnarumma *et al.*, 2014; Goodwin *et al.*, 2014) communities. A striking pattern of community change over these pH gradients consistently is a shift away from calcifying taxa (e.g. coralline algae, molluscs) towards non-calcified species (e.g. fleshy brown algae, anemones). These patterns are driven by a combination of direct effects, such as the dissolution of calcareous shells/skeletons combined with higher energetic costs associated with calcification (Wittmann & Pörtner, 2013), and effects mediated by species interactions such as changes in competition, predation and habitat structure (Connell *et al.*, 2013; Kroeker *et al.*, 2013b; Linares *et al.*, 2015; Sunday *et al.*, 2017). Although community-level outcomes (and the species interactions that underlie them) have been documented for a wide range of marine communities, the effects of $p\text{CO}_2$ on recruitment, succession and development have been mainly investigated in algal communities (Kroeker *et al.* 2011; 2012; 2013b) and similar studies are lacking for marine invertebrates (although see Brown *et al.*, 2016). Furthermore, the effects of the timing and duration of acidification events during succession have seldom been addressed, although the response of marine communities to acidification has been shown to change with seasonality (e.g. Baggini *et al.*, 2014) and with timing and length of upwelling events (Iles *et al.*, 2012).

At CO_2 seeps, within-seep vs. outside-seep recruitment is difficult to disentangle and life history strategy may determine the extent of direct effects of acidification on species-specific recruitment. If recruits are coming from within-seep source populations, which is most likely for species with short pelagic larval phases, observed recruitment effects (both positive and

negative) could represent a culmination of both direct effects of acidification on larvae (e.g., physiological, Kurihara *et al.*, 2008; Ross *et al.*, 2011; Przeslawski *et al.*, 2015; and/or behavioural Doropoulos *et al.*, 2012; Doropoulos & Diaz-Pulido, 2013; Webster *et al.*, 2013), transgenerational effects of acidification on nearby adult populations (Calosi *et al.*, 2013a; Harvey *et al.*, 2016; Ross *et al.*, 2016), and multigenerational adaptation to chronic acidified conditions (Calosi *et al.*, 2013a). In contrast, propagules arriving from outside the seeps will not experience generational effects of acidification.

In this study, we observed how benthic marine invertebrate communities recruited and developed along a natural $p\text{CO}_2$ gradient. We used reciprocal transplant experiments to determine if fouling communities would reflect a lasting response to short but discrete exposure to elevated $p\text{CO}_2$ (e.g. during an upwelling event) in early succession or reflect only most recent exposure to elevated $p\text{CO}_2$ regardless of prior conditions. We expected that $p\text{CO}_2$ would disrupt succession by altering recruitment of primary vs. secondary colonizers and alter the interactions among these taxa via inhibition or facilitation (Connell & Slatyer, 1977). We predicted that the relative importance of discrete exposure early on in succession, continuous exposure throughout succession or exposure later in succession on a given species would depend on life history traits of species. For example, if timing of recruitment coincides with timing of acidification, a discrete exposure to CO_2 that occurs early on in succession may influence primary colonizers more than secondary colonizers. Other factors, like environmental tolerances, growth rate, ability to induce a resting stage, and concentration of propagules may determine how a given taxa responds to and/or rebounds from a short and discrete CO_2 event. At the community level, we hypothesized that acidification would result in homogenized, low-diversity communities of biofouling organisms, dominated by a few weed-like species with notable reductions in calcified taxa.

Materials and Methods

Site description and experimental set up

The study was conducted in Levante Bay on Vulcano Island (38°25'08"N, 14°57'40"E) in the Aeolian archipelago in Northeastern Sicily (Italy) from March to June, 2013 (12 weeks). At this site, shallow volcanic seeps emit carbon dioxide bubbles that create a gradient in seawater carbonate chemistry that has been characterized by a number of recent studies (Arnold *et al.*, 2012; Johnson *et al.*, 2012; Lidbury *et al.*, 2012; Boatta *et al.*, 2013; Calosi *et al.*, 2013b; Milazzo *et al.*, 2014). The biogeochemistry of the bay has been assessed to identify the most suitable areas for ocean acidification research (see Boatta *et al.*, 2013; Vizzini *et al.*, 2013) and the chosen sites were outside of any measured metal contamination. Variability in the pH gradient at the site is predominantly driven by currents influenced by westerly winds, and as such, the acidified water masses mostly run parallel to the northern shoreline of the bay (Boatta *et al.*, 2013). When winds are high, e.g. during storms that are common in winter and early spring, low pH waters are more restricted to the immediate vicinity of the seeps and do not extend as far along the shoreline (Boatta *et al.* 2013). We used two sites, ~70 m apart, along the $p\text{CO}_2$ gradient; the first had low mean pH (7.78), and the second had ambient mean pH (8.10) (here we use high $p\text{CO}_2$ and low pH interchangeably). Our sites correspond to sites 40-60 and 120-130 in Boatta *et al.*, (2013) for low pH and control pH respectively. We monitored temperature, salinity, and pH at each site at least once every two weeks throughout the observation period but daily for the first week and the last six weeks of the experiment. Samples for total alkalinity were taken every two weeks. We used these parameters to calculate dissolved inorganic Carbon (DIC), $p\text{CO}_2$, HCO_3^- , and carbonate and aragonite saturation states using the CO2-SYS program (Pierrot *et al.* 2006).

At each site, we suspended 70.5×70.5 cm semi-flexible PVC panels from buoys 1 m from the surface and 1 m from the bottom ($n=3$ panels per site). We deployed the panels on similar substrata at each site, which comprised of subtidal boulders and patches of seagrass. Each panel was oriented horizontally (Fig 1), and 15 PVC tiles (14.5 cm x 14.5 cm) were secured to the underside of each panel using cable ties ($n=15$ tiles nested within each of 3 panels per site, therefore $n=45$ tiles per site). The panels were secured to the buoys and anchors using ropes to avoid horizontal spinning. Storms damaged some tiles and panels, so only undamaged tiles ($n=20$ per site, from across the three panels) were used in the analyses. After 8 weeks, we reciprocally transplanted a subset of 10 tiles (selected randomly from the 20 total) from each $p\text{CO}_2$ regime, while the other 10 tiles were not transplanted, to determine if $p\text{CO}_2$ effects on recruitment occur early and persist or arise later during succession. At the time of this transplantation, we formed new panels (2 per site) with ten tiles each, tiles from transplanted and non-transplanted groups were distributed randomly to the two panels in each site.

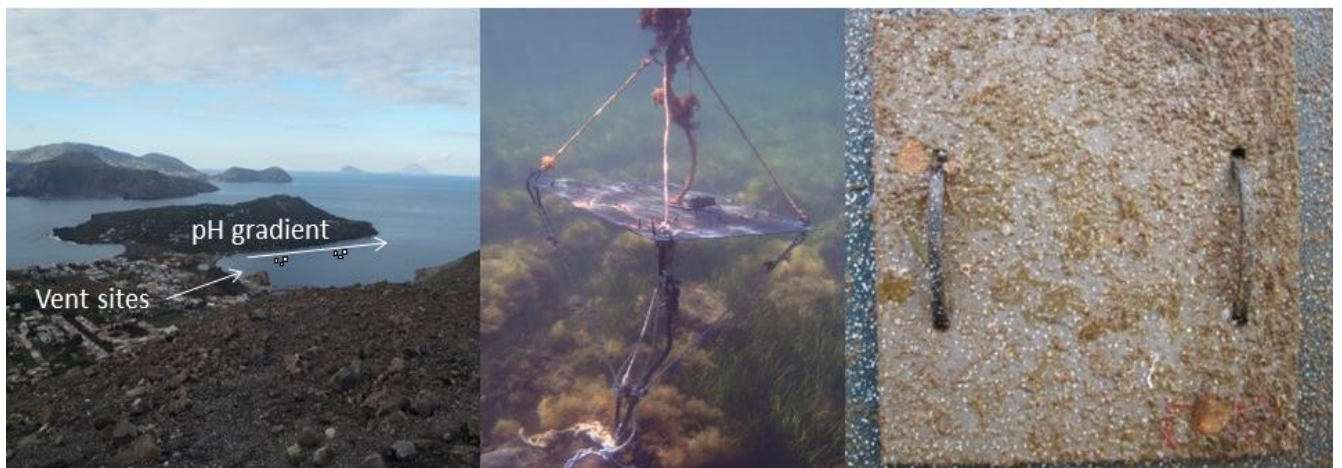


Fig. 1. Photographs depicting sites (white symbols) along the pH gradient (left) and the panel and tile system. Panels (centre), with downward facing PVC tiles (right) attached to the underside, were suspended ~1m from both surface and bottom using a buoy and anchor.

Species- and community- level measures

Photographs (one per tile) were taken every two weeks to determine changes in community composition, structure, Shannon's diversity, richness, stability over time and percent cover (point count) of primary and secondary colonizing fouling species for eight weeks. After transplantation, the tiles were left for one additional month before being photographed and retrieved for preservation. The tiles and panels were brought to the surface for photography. We conducted all photographic analyses in ImageJ (Schneider *et al.*, 2012) and identified species to lowest taxonomic level possible in the laboratory. Primary colonizers were defined as those that recruited in the ambient site in the first four weeks, before the community reached 100% cover. Primary colonizers included two serpulid polychaete taxa (serpulidae, spirorbidae) and two algal guilds, a turf-forming green filamentous alga *Cladophora* sp., and a biofilm which was ubiquitous and has been described at this site as primarily a mix of diatoms and cyanobacteria, the composition of which changes along gradients of $p\text{CO}_2$ (Johnson *et al.*, 2015). Secondary colonizers (i.e., those that recruited only after space occupancy reached 100% at week four) included bryozoans (e.g. *Schizomavella* sp. and *Patinella radiata*), ascidians (*Botryllus* sp. and *Diplosoma* sp.), and *Sphacelaria* sp., which is a branched red alga. These secondary colonizers may require a layer of biofilm in order to recruit, or their propagules may have only be in the water column during the latter half of the experiment due to seasonal or episodic reproductive patterns. Natural succession in marine communities often coincides with a disturbance regime (e.g. winter storms, sedimentation) that creates space (Sousa, 1979). The disturbance regime at our study site may coincide with seasonality, and we expect that the succession observed from bare surfaces reflected both directional community development and response to warming temperatures over time.

204 *Statistical analyses*

205 All statistical analyses were performed using open-source R (R Development Core Team, 2009).
206 We used the *adonis* and *Betadisper* functions in the *Vegan* package (Oksanen *et al.*, 2015) to
207 analyze multivariate community structure (PERMANOVA test) and homogeneity of dispersions,
208 respectively. Community structure analyses were conducted on species abundance data (percent
209 cover) of a given tile, standardized by total abundance of that species across all tiles, putting
210 abundances of ecologically different species on the same scale. This community structure metric
211 was calculated twice: pre-transplant using data from week 8, and post-transplant using data from
212 week 12. We then calculated a Bray Curtis dissimilarity matrix on the standardized data. In all
213 tests, for a given week, we used site as a fixed factor and separately tested for the effect of panel
214 within site, and if initial or final panel (nested within initial or final site, respectively) had a
215 significant effect, we included the term in the full model. To test if acidification influenced
216 community structure at week 12 we used the 10 non-transplanted replicates per site. Next, to
217 analyze if, during the reciprocal transplant, initial or final site or their interaction influenced
218 community structure, we used all 20 tiles (10 of which had been transplanted from the other site).
219 We calculated pre- and post-transplant community stability, the temporal mean over temporal
220 variability of species abundances in a given tile, using the *community.stability* function in *codyn*
221 package (Hallett *et al.*, 2016).

222 We used both linear mixed effects models (LMEs, *lme4* package, Bates *et al.*, 2015) and
223 generalized liner mixed-effects models (GLMMs, *glmmADMB* package, Fournier *et al.*, 2012;
224 Skaug *et al.*, 2013) to analyze percent cover and count data (from photographs), community
225 stability, and proportion of secondary colonizers. For the first 8 weeks (pre-transplantation), we
226 used site as a fixed effect (n=20 per site), after which (post-transplantation) we used initial site,

final site, and their interaction as fixed effects to incorporate tiles that were reciprocally transplanted (n=10 per site) and those that were not (n=10 per site). For a given point in time, tile was the level of replication and we treated both initial and final panels nested within site as random factors and all models included a random intercept. Numerical response variables (species richness, cumulative counts) were considered either normally distributed and analyzed with LMEs or Poisson or negative binomially distributed and analyzed with GLMMs, to assess effect of site, depending on distribution fit to the data. We assumed either a Beta or Binomial distribution (based on fit of distribution to data) for percent cover data and analyzed the effect of site with GLMMs. We used a GLMM with assumed Gamma distribution to analyze the effect of acidification on Shannon's diversity.

Results

Seawater parameters

Seawater pH fluctuated with time of day and wind direction. However, pH was consistently lower in the low pH site compared to the ambient site ($\Delta\text{pH}_{\text{NBS}} = -0.32 \pm 0.19$, mean \pm SE, daily differences between sites averaged across experimental period, Table 1, LM, $F=141.7$, $P<0.0001$). Total alkalinity was consistent across sites at a given time point ($\Delta\text{TA} = 3.45 \pm 1.38$, Table 1, LM, $F=3.17$ $P=0.08$). During the experimental period, temperatures ranged from 14.4°C to 20.8°C and salinity from 37.8 to 38.6. Differences in temperature ($\Delta\text{temperature} = 0.01^\circ\text{C} \pm 0.15^\circ\text{C}$, LM, $F=0.0019$, $P=0.97$) and salinity ($\Delta\text{salinity} = 0.00 \text{ ppt} \pm 0.01 \text{ ppt}$, LM, $F=0$, $P=1$) were negligible between sites during the survey.

Table 1. Carbonate chemistry of seawater from ambient and low pH sites. Temperature, salinity, pH_{NBS}, and total alkalinity were collected from March to June 2013 (mean ± SE, n = 98). Asterisks indicate calculated values in the CO2-SYS program (Pierrot et al. 2006).

Seawater parameter	Control	Low pH	
Temperature (°C)	18.96±0.15	18.97±0.15	256
Salinity	38.19±0.011	38.19±0.010	257
pH _{NBS}	8.10±0.13	7.78±0.24	258
Alkalinity (μmol kg ⁻¹)	2523.52±1.34	2526.97±1.41	259
pCO ₂ (μatm)*	557.69±26.48	1499.91±151.71	260
DIC (μmol kg ⁻¹)*	2271.16±6.42	2424.09±10.38	261
HCO ₃ ⁻ (μmol kg ⁻¹)*	2066.59±9.62	2270.29±10.15	262
Ω Calcite*	4.33±0.096	2.43±0.093	263
Ω Aragonite*	2.82±0.063	1.59±0.060	

Primary colonization pre- and post-transplant

All tiles were colonized initially by biofilm. The biofilm grew more rapidly, and peaked and declined in cover earlier on tiles at the acidified site relative to the ambient site. After eight weeks, biofilm cover was higher on tiles at the acidified site than the ambient site (Fig. 2a; statistics are summarized in Table 2). Filamentous alga, *Cladophora* sp., had higher cover at the acidified site at its peak, although by week 8 there was no difference in cover between sites (Table 2, Fig. 2c). Calcified polychaetes – serpulid and spirorbid worms – had at least a two-week delay in recruitment at the acidified site, and, by week 8, covered significantly less space (Table 2, Fig. S1a, c) and had less than a third the density at the acidified site relative to the ambient site (Table 2, Fig. 2e, g). Both serpulids and spirorbids would be considered primary colonizers under ambient conditions but classified as secondary colonizers (arriving after 100% space occupancy had been reached) under acidified conditions.

276 **Table 2.** Statistical results from both GLMM (using z statistic) and LME (using X^2) from analysis of percent cover
 277 of a given species. ^p indicates week in which peak % cover of this species occurred.

Group	Species	Abundance measure	Week 8 (P=peak)	Mean abundance ambient site	Mean abundance low pH site	z or X^2 *	P * <0.05
	Biofilm complex	% cover	8	85.6	93.1	-4.43	<0.0001*
	<i>Cladophora</i> sp.	% cover	8	0.050	0.62	-1.44	0.15
	Serpulids	% cover	8 ^p	0.52	0.30	3.33	<0.001*
		# individuals		3.4	1.0	4.81	<0.001*
	Spirorbids	% cover	8 ^p	7.06	2.05	3.05	<0.001*
		# individuals		572.3	129.1	14.4*	<0.001*
	<i>Diplosoma</i> sp.	% cover	8	5.0	2.0	1.79	0.074
		# colonies		2.0	0.4	5.22*	0.022*
	<i>Botryllus</i> sp.	% cover	8	0.44	0.69	-1.95	0.051
		# colonies		1.9	2.8	-1.30	0.19
	Thin ramified bryozoan	% cover	8	0.69	0.07	2.57	0.01*
	<i>Patinella radiata</i>	% cover	8	0.050	0.074	-0.19	0.85
		# colonies		2.2	2.8	-0.30	0.76
	<i>Schizomavella</i> sp.	% cover	8	0.15	0.025	0.98	0.33
		# colonies		1.2	0.2	2.35	0.0019*

278 Eight weeks into the experiment, a subset of tiles was reciprocally transplanted among sites. One
 279 month after transplantation, biofilm cover was related to only the most recent exposure to $p\text{CO}_2$
 280 (i.e. tiles maintained in or transplanted to low pH) as coverage was lower on tiles that ended up
 281 at the acidified site than those in the ambient site, (GLMM, Final site $P=0.050$, Table 3, Fig.2b),
 282 regardless of origin, and there was no evidence of the pre-transplant effects of $p\text{CO}_2$ carrying
 283 over (GLMM, Initial site $P=0.69$, Table 3, Fig.2b). *Cladophora* sp. cover was reduced to zero
 284 after 8 weeks, therefore we were unable to determine if $p\text{CO}_2$ was more important in early or late
 285 succession for this taxon. Transplant results suggested that serpulid recruitment was influenced
 286 by $p\text{CO}_2$ early on and persisted, although there were also $p\text{CO}_2$ effects present during late
 287 succession. Overall, tiles that originated at the ambient site recruited more serpulid individuals

288

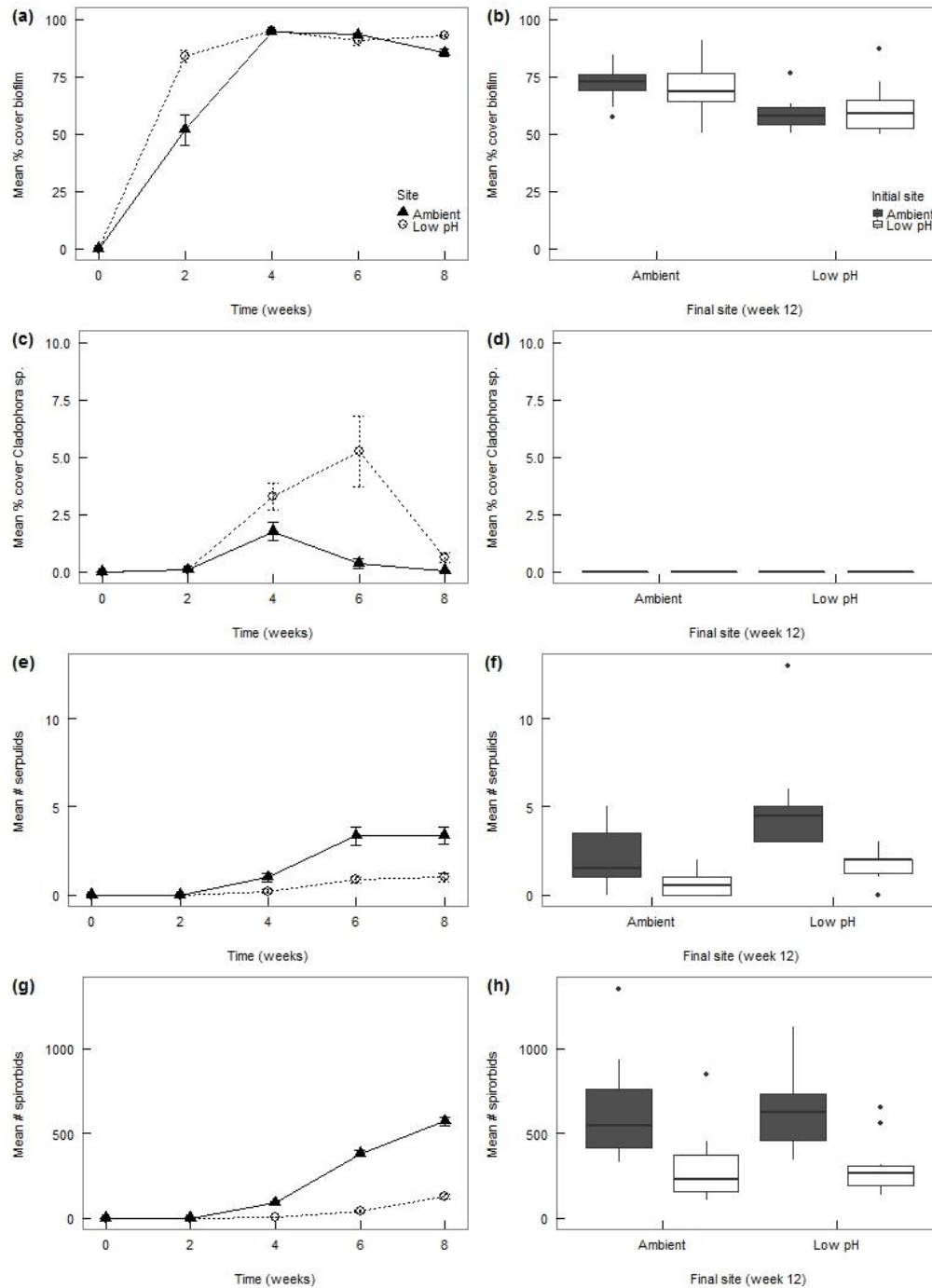


Fig. 2. Abundance of selected primary colonizers in ambient and low pH sites over time. Left-hand panels are trends through week 8 (i.e., pre-transplantation; n=20) and right-hand panels are patterns at week 12 (post-transplantation; n=10) of both transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis indicates final site. Species are: (a, b) biofilm (% cover), (c, d) *Cladophora* sp. (% cover), (e, f) serpulids (# individuals) and, (g, h) spirorbids (# individuals). Error bars indicate standard error.

Table 3. Results of GLMMs using percent cover of a given species and initial site, final site and their interaction as fixed effects (n=20).

Group	Species	Initial site		Final site		Initial site * Final site	
		<i>z</i>	<i>P</i> * <i><0.05</i>	<i>z</i>	<i>P</i> * <i><0.05</i>	<i>z</i>	<i>P</i> * <i><0.05</i>
	Biofilm complex (% cover)	-0.40	0.69	-1.96	0.050*	0.49	0.62
	<i>Cladophora</i> sp. (% cover)	0.0	1.0	0.0	1.0	0.0	1.0
	Serpulids (% cover)	-1.83	0.067	3.06	0.0022*	-0.65	0.52
	(# individuals)	-2.51	0.012*	3.19	0.0014*	0.22	0.83
	Spirorbids (% cover)	-0.79	0.43	0.81	0.42	-0.47	0.64
	(# individuals)	-2.33	0.02*	0.46	0.65	-0.17	0.87
	<i>Diplosoma</i> sp. (% cover)	0.13	0.89	0.80	0.43	1.68	0.093
	(# colonies)	-1.62	0.11	-0.80	0.42	0.82	0.41
	<i>Botryllus</i> sp. (% cover)	0.49	0.63	1.19	0.26	-0.48	0.63
	(# colonies)	-0.02	0.99	0.27	0.79	0.46	0.4
	Thin ramified bryozoan (% cover)	0.30	0.77	3.41	<0.001*	-2.21	0.027*
	<i>Patinella radiata</i> (% cover)	-0.20	0.84	1.38	0.17	-0.49	0.62
	(# colonies)	-0.04	0.97	-0.53	0.60	0.58	0.56
	<i>Schizomavella</i> sp. (% cover)	-2.59	0.0096*	-0.70	0.49	0.29	0.77
	(# colonies)	-0.34	0.73	0.20	0.84	-0.31	0.76

298

299 than those that originated at the acidified site, regardless of final site (GLMM, Initial site
300 $P=0.012$, Table 3, Fig. 2f) and cover of this species showed a non-significant trend in the same
301 direction (Initial site $P=0.067$, Table 3). However, by the end of the experiment, there were more
302 individuals and higher cover of serpulids on tiles that had final exposure to high $p\text{CO}_2$, rather
303 than ambient conditions (GLMM, Final site $P=0.0014$, Table 3, Fig. 2f, Fig. S1b). Initial site
304 alone influenced the number of individuals, but not cover, of spirorbids, such that tiles that
305 originated in the ambient site had more spirorbids, regardless of their final locations (GLMM,
306 Initial site $P=0.02$, Table 3, Fig. 2h, Fig. S1d).

307 *Secondary colonization pre- and post-transplant*

308 Fewer colonies of the colonial ascidian, *Diplosoma* sp., recruited by week 8 at the acidified site
309 (Table 2, Fig. S1e) but these colonies covered a similar amount of space in both sites (Table 2,
310 Fig. 3a). At the same time point, another colonial ascidian, *Botryllus* sp., had similar cover
311 between sites (Table 2, Fig. 2c) and no difference in number of recruiting colonies between sites
312 (Table 2, Fig. S1g). Bryozoans, a phylum with a broad range of morphologies, yielded mixed
313 responses to acidification. At week 8, an erect calcitic bryozoan, *Patinella radiata*, had both
314 similar cover (Table 2) and number of colonies at the acidified site compared to the ambient site
315 (Table 2, Fig. S1i). Thin ramified bryozoan had higher cover under ambient conditions while
316 essentially absent from the acidified site (Table 2, Fig. 3e), while the encrusting aragonitic
317 bryozoan, *Schizomavella* sp. recruited fewer colonies on tiles at the acidified site compared to the
318 ambient site (Table 2, Fig. 3g) but had similar cover (Table 2) between sites at week 8.

319 After the transplant experiment, soft-bodied ascidians appeared to be largely resistant to changes
320 in acidification. Neither initial site nor final site influenced cover or number of *Diplosoma* sp. or
321 *Botryllus* sp. (Fig. 3b,d, Fig. S1f,h Table 3). The bryozoan *P. radiata* remained resistant to
322 acidification after transplantation, and there was neither an effect of initial nor final site on
323 numbers of colonies and cover of this species (Table 3). Post-transplant thin ramified bryozoan
324 cover was influenced by final site, as overall cover was higher on tiles that ended up at the
325 acidified site (Final site $P<0.001$), and this effect was especially strong for tiles transplanted
326 from the ambient site (Initial*final site $P=0.027$, Table 3, Fig. 3f). Early successional $p\text{CO}_2$
327 effects were apparent in post-transplant recruitment of *Schizomavella* sp., such that tiles that
328 originated in the ambient site had more colonies than those from the low pH site (Initial site
329 $P=0.0096$, Table 3, Fig. 3h).

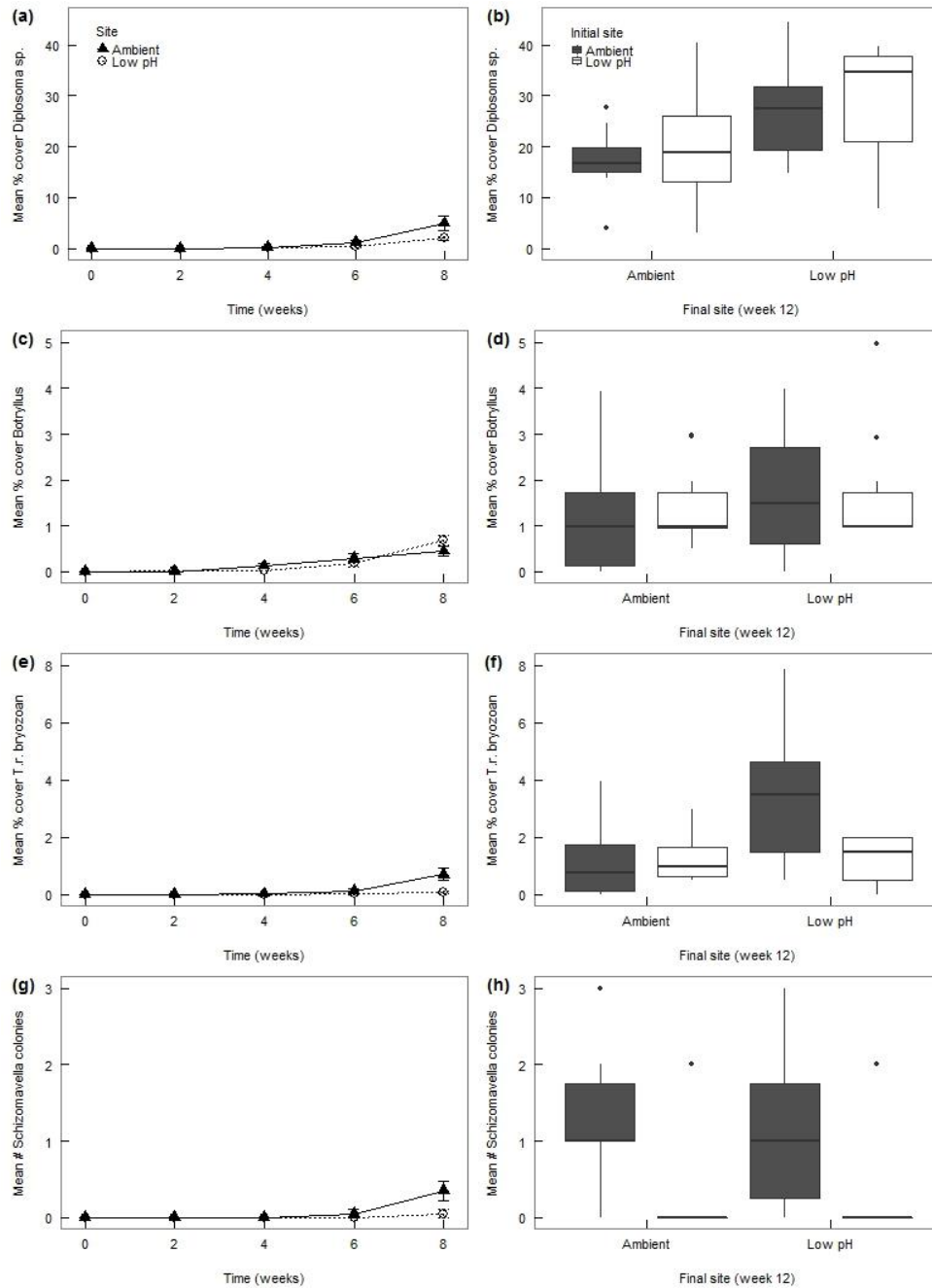


Fig. 3. Abundance of selected secondary colonizers in ambient and low pH sites over time. Left- and right-hand panels as in Figure 2. Species are: (a, b) *Diplosoma* sp. (% cover), (c, d) *Botryllus* sp. (% cover), (e, f) Thin ramified bryozoan (% cover) and, (g, h) *Schizomavella* sp. (# colonies). Error bars indicate standard error.

Community level results pre- and post-transplant

The above changes in species recruitment and succession culminated in significant shifts in community structure on tiles at the acidified site at week 8 (PERMANOVA, $R^2=0.16$, $P=0.0001$, Fig. 4a). Although community structure differed, there was no difference in community variance between sites (Betadisper, $F=0.63$, $P=0.80$, Fig. 4a). Tiles were colonized more quickly under acidified conditions than those at the ambient site but this difference was only apparent for the first four weeks, and the tiles had similar cover at the 8th week (LME, $X^2=2.16$, $P=0.14$).

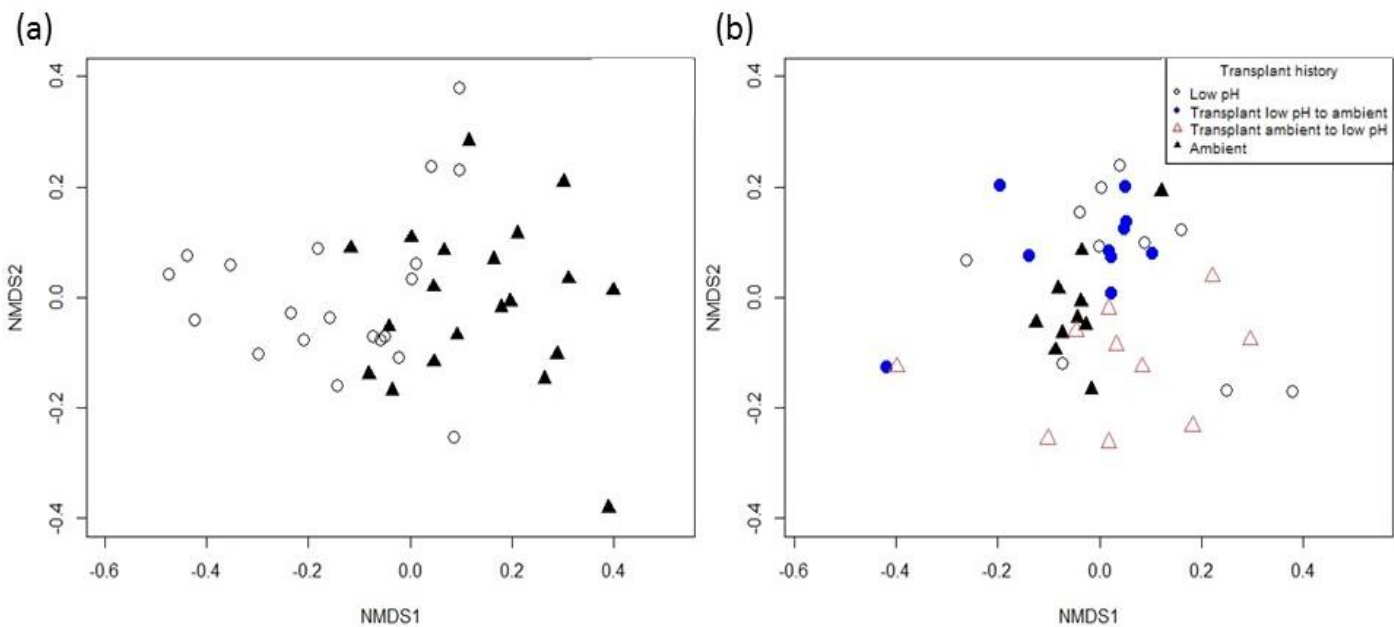
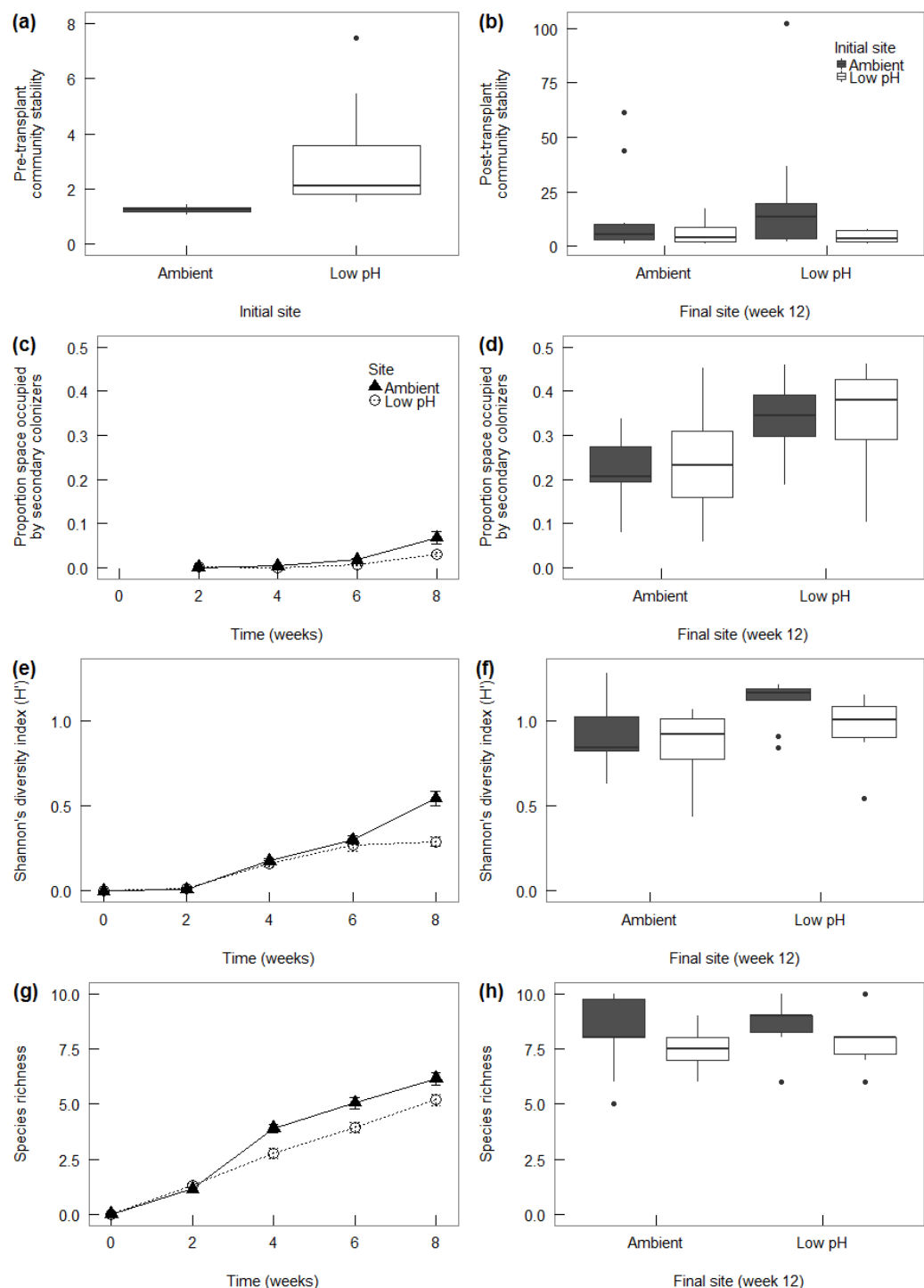


Fig. 4. nMDS ordination plot showing the relationship between communities after (a) 8 weeks on tiles from low pH (open circles) vs. the ambient site (solid black triangles), $n = 20$ tiles, and (b) 12 week on tiles that either remained in low pH (open circles), were transplanted from low pH to the ambient site (solid blue circles), were transplanted from ambient site to low pH site (red open triangles), or remained in the ambient site (solid black triangles), $n = 10$ tiles.

Pre- transplant community stability was higher for tiles at the acidified site than the ambient site (LME, $X^2=19.5$, $P<0.0001$, Fig. 5a), indicating that over this time period, communities at the ambient site changed more than those at the acidified site. Secondary colonizers, which arrived after the 4th week, initially gained cover at the same rate in both sites but by the 8th week took up more space at the ambient site than at the acidified site (LME, $X^2=4.04$, $P=0.044$, Fig. 5c), indicating that succession from primary to secondary species occurred earlier at the ambient site. We observed negative effects of acidification on diversity (GLMM, $z=3.41$, $P=0.00065$, Fig. 5e), but species richness was similar between sites (GLMM, $z=1.25$, $P=0.21$, Fig. 5g) after 8 weeks.

After transplantation, both initial site and final site influenced community structure, i.e. communities that originated at the ambient site were different overall than those that originated at the acidified site (PERMANOVA, initial site, $R^2=0.10$, $P=0.0001$, Fig 4b: triangles vs. circles) and communities that ended at the acidified site differed from those that ended at the ambient site (PERMANOVA, final site, $R^2=0.053$, $P=0.013$, Fig. 4b: open vs. solid symbols). There was no evidence of an interaction between the effects of pCO_2 on early and late succession (PERMANOVA, initial*final site, $R^2=0.027$, $P=0.26$, Fig. 4). In addition, although there was no evidence that pCO_2 affected community variability early in succession (Betadisper, initial site, $F=0.14$, $P=0.71$, Fig 4: triangles vs. circles), there was an influence of final site on variability – as tiles that were transplanted to the high pCO_2 site were significantly less variable than those that ended at the ambient site (Betadisper, final site, $F=8.04$, $P=0.0073$, Fig.4: open symbols are more dispersed than solid symbols). Community stability between the 8th and 12th week was similar between sites, regardless of transplantation history (LME, final site, $X^2=0.0001$, $P=0.99$,

373 initial site: $X^2=2.32$, $P=0.13$, initial*final site: $X^2=0.94$, $P=0.33$, Fig. 5b).



375 **Fig. 5.** Community-wide measures in ambient and low pH sites over time, left- and right-hand
 376 panels as in Figure 2. Measures are: (a, b) community stability, (c, d) secondary colonizers space
 377 occupation, (e, f) Shannon's diversity, and (g, h) species richness. Error bars indicate standard
 378 error.

However, the proportion of secondary colonization was higher on tiles that ended under low pH conditions, regardless of origin (GLMM, final site, $z=2.63$, $P=0.20$, initial site: $z=0.33$, $P=0.75$, initial*final site: $z=-0.26$, $P=0.80$, Fig. 5d). After transplantation, Shannon diversity was significantly higher on tiles that ended at the elevated $p\text{CO}_2$ site (GLMM, final site: $z=2.23$, $P=0.026$, Fig. 5f), while the negative effects of $p\text{CO}_2$ observed during early succession appeared to have no persisting influence on diversity by the end of the experiment (GLMM, initial site: $z=-0.69$, $P=0.49$, initial*final site: $z=-0.58$, $P=0.56$). Species richness appeared resistant to acidification after the transplantation experiment, as neither early nor late $p\text{CO}_2$ effects influenced the number of recruiting species (GLMM, initial site: $z=-0.47$, $P=0.64$, final site: $z=0.31$, $P=0.76$, initial*final site: $z=-0.10$, $P=0.92$, Fig. 5h).

Discussion

Timing and abundance of species recruiting from plankton are important determinants of long-term community composition and structure (Sutherland, 1974; Sams & Keough, 2012) and, depending on the mechanism of succession, can determine long-term community stability (Connell & Slatyer, 1977). Environmental heterogeneity and disturbance regimes during recruitment can influence successional outcomes by promoting coexistence or dominance of early or late recruiting species (Platt & Connell, 2003; Cifuentes *et al.*, 2010). Global change impacts on communities and ecosystems may therefore stem from the particular way in which environmental drivers interact with life-history trade-offs (e.g. competitive ability vs. dispersal / colonization ability) of early and late successional species, and how these species inhibit or promote one another. This is reasonably well understood for drivers like temperature in terrestrial ecosystems (e.g. Gounand *et al.*, 2016; Lancaster *et al.*, 2016), but in marine

environments and for emerging drivers like ocean acidification, changes in succession – even when observed (e.g. Kroeker *et al.*, 2013b) – are rarely explicitly examined in terms of the underlying mechanisms and time-history contingency.

Here, we used transplant experiments to elucidate the relative importance of $p\text{CO}_2$ effects early vs. later in succession on species abundance, diversity and composition. This is important for understanding how acidification might affect dynamic communities as they progress through successional and seasonal development. It is also key for determining the effects of discrete acidification events on marine communities. Upwelling regions experience intermittent acidification events that can span weeks (e.g. up to six week periods, Chan *et al.*, 2017) or months (e.g. from early spring to late summer, Feely *et al.*, 2008) and these events are expected to become less frequent but longer-lasting and stronger (Iles *et al.*, 2012). Acidification events also occur in areas with high organic loading via terrestrial run-off, where discrete events can last for days to weeks but multiple cumulative events can occur over several months (Guadayol *et al.*, 2009). Eutrophication-driven acidification may intensify in the future as eutrophication will likely increase with increased human development (Rabalais *et al.*, 2009).

On our experimental tiles, soft-bodied, weed-like taxa, algae and ascidians, had an advantage in acidified conditions and outcompeted calcified taxa that were more vulnerable to the effects of acidification, as has been widely reported (Wittmann & Pörtner, 2013). Developing communities responded quickly to acidification (effects on some species were apparent after two weeks) and some of these effects were not reversed one month after transplantation. In sum, we found that succession was substantially altered by acidification, even when acidified conditions were not maintained for the full duration of the experiment. Future work should carefully consider

temporal variation in acidification over experimental periods spanning important dynamics in community succession.

Recruitment and development

Early successional stages in many shallow benthic habitats – including, to our slight surprise, downward-facing experimental surfaces – are dominated by photosynthetic microalgae and weedy macroalgae. Many photosynthetic marine taxa can take advantage of elevated $p\text{CO}_2$ (Connell *et al.*, 2013; Cornwall *et al.*, 2017). At Vulcano Island and other seep sites, biofilms are higher in percent cover and productivity and have altered composition relative to reference sites (Lidbury *et al.*, 2012; Johnson *et al.*, 2013, 2015; Baggini *et al.*, 2015). We observed the same boost in biofilm under acidified conditions during the early phase of succession, although it is unclear if composition changed. It is possible that the biofilm at low pH sites altered subsequent invertebrate recruitment by changing settlement cues (see Doropoulos *et al.*, 2012), as succession was delayed in this site, despite early biofilm abundance. Filamentous green *Cladophora* sp. had a higher and slightly later peak in abundance on tiles at the acidified site. Such shifts in primary producer assemblages alter biomass of resources available for grazers (Russell *et al.*, 2013) and may alter settlement patterns of recruiting invertebrates (Hadfield, 2011).

We found that calcified primary colonizers had lasting responses to short but discrete exposure to increased $p\text{CO}_2$, whereas only one of the calcified secondary colonizers exhibited this response. This pattern could be caused by the differential duration of exposure experienced by primary (four weeks longer) vs. secondary species or traits of the species in each of the categories (e.g. primary colonizers were heavily calcified polychaetes, compared to relatively lightly calcified bryozoans). The recruitment of two types of calcified tube-forming polychaetes was both reduced and delayed under acidification. Delayed recruitment under acidification causes

individuals of these species to arrive after the community has reached 100% cover and could imply that these organisms face stiffer competition for space than their counterparts that recruited earlier into the ambient site. Tile site of origin influenced the recruitment of both spirorbids and serpulids and these effects persisted through time, regardless of transplantation into or out of the acidified site. Our results align with observations of reduced abundances of both serpulids and spirorbids near CO₂ seeps off Ischia (Cigliano *et al.*, 2010; Donnarumma *et al.*, 2014), but suggest that this effect emerges very early in the establishment of these taxa. The two-week delay in recruitment could be due to a combination of direct effects on adult reproduction, larval and juvenile recruitment and/or indirect effects such as inhibition by settled species. Acidification has been shown to impair serpulid larval calcification and juvenile growth (Lane *et al.*, 2013) and compromise tube ultrastructure (Li *et al.*, 2014) in the laboratory. Spirorbid growth could have been influenced by early exposure to *p*CO₂, as there were fewer spirorbids on tiles that originated in acidified sites, but similar space coverage, suggesting that spirorbids under acidification were larger. This could be a consequence of accelerated growth under acidification or differential mortality of smaller individuals within the population. Negative effects of acidification on these polychaetes may have higher level consequences as these ecosystem engineers form complex reefs that have high associated biodiversity (Smith *et al.*, 2013; Fabricius *et al.*, 2014).

At various points of succession, colonial ascidians (*Diplosoma* sp. and *Botryllus* sp.) appeared either to tolerate or respond positively to acidification, which may reflect increased growth rate, increased facilitation, and/or reduced competition under increased *p*CO₂ conditions. It is difficult to disentangle these effects as growth rate in this context is undoubtedly influenced by other species, and facilitation and competition is difficult to infer without experimental manipulation.

470 These ascidians, although native to the Mediterranean, are among a suite of globally invasive
471 taxa that overgrow other filter feeders and can cause economic damage to the aquaculture
472 industry (Zhan *et al.*, 2015). Our results add to growing evidence that some ascidians respond
473 positively to both natural (Donnarumma *et al.*, 2014) and experimental (Peck *et al.*, 2015)
474 acidification (but see Fabricius *et al.* (2014) for an example of reduced ascidian cover at tropical
475 seep sites). Overall, fast-growing nuisance species like ascidians are expected to benefit from
476 future acidification (Hall-Spencer & Allen, 2015) and reduced competition with calcifying native
477 taxa might increase relative dominance of invasive ascidians in an acidified ocean.

478 We observed mixed effects of natural acidification on lightly calcified bryozoans, which may be
479 related to differences in their carbonate mineralogy. The cyclostome bryozoan *Patinella radiata*,
480 with a primarily calcitic skeleton, did not change in abundance near seep sites. Studies off Ischia
481 have shown that this species can grow and reproduce at low pH (Donnarumma *et al.*, 2014;
482 Taylor *et al.*, 2015). However, a thin ramified bryozoan appeared earlier at the ambient than the
483 acidified site and an encrusting bryozoan *Schizomavella* sp., with a mainly aragonitic or
484 bimineralic skeleton (Smith *et al.*, 2006), had reduced recruitment at the acidified site. Carbonate
485 mineralogy of bryozoans can help predict vulnerability to acidification for a given species -
486 aragonite skeletons are more soluble than those composed of mainly calcite, and calcite
487 solubility increases with proportion of Mg (Fortunato, 2015; Pickett & Andersson, 2015; Taylor
488 *et al.*, 2015). However, mineralogy is not the sole determinant of dissolution rate. Other factors
489 such as surface area, porosity, surface complexity, organic matrix material, and ambient pH at
490 the calcification surface, may also play a role in response to acidification (Ries *et al.*, 2009;
491 Smith & Garden, 2013; Taylor *et al.*, 2015). Morphological differences between related species
492 highlight the importance of examining species-specific responses to acidification. However, the

relevance of these morphological differences among species may be outweighed by relative competitive ability if and when these bryozoans are at risk of being overgrown by other species.

Species interactions and community-level results

Acidification first delayed, then accelerated community succession. First, communities in acidified sites developed more slowly (i.e. were more stable) than ambient communities and had a smaller proportion of space used by secondary colonizers. After 8 weeks, however, the acidified communities developed rapidly and rate of community development was similar between acidified and ambient communities. At this point, the proportion of secondary colonizers increased on tiles at the high $p\text{CO}_2$ site, independent of colonization history. This mismatch in timing indicates that ocean acidification may alter species interactions between and within primary and secondary colonizer guilds. For example, the biofilm trajectory, likely a diatom bloom, was altered at the acidified site, resulting in reduced biofilm coverage at the acidified site by the end of the experiment. This likely contributed to increased cover of serpulids and bryozoans on tiles that experienced higher $p\text{CO}_2$ at the acidified site since, at ambient levels of $p\text{CO}_2$, the abundant biofilm overgrew and/or pre-empted space occupation by these invertebrates, compared to the acidified site where there were fewer calcified invertebrates and lower biofilm cover during this time period. The benefits to the calcifying organisms transplanted to the acidified site may be short lived however, as some of these species were negatively affected by acidification overall.

Accelerated succession (despite an initial delay) and competition-mediated reductions in invertebrates at the ambient site likely contributed to higher species diversity on tiles that were maintained in or transferred to the acidified site. This pattern was not observed for species richness however, indicating that evenness or abundance of species was driving the diversity

result. This unexpected result further underscores the importance of understanding shifting species interactions under acidification (Gaylord *et al.*, 2015). Responses to $p\text{CO}_2$ could also be modulated at seep sites by seasonal effects on both calcifying invertebrates and algal competitors (Baggini *et al.*, 2014), as seawater temperature increased from 14°C to 20°C during our experiment. Competition between serpulids and algae has been documented in benthic communities near Ischia CO_2 seeps, although the pattern described there (Kroeker *et al.*, 2013b) is opposite to what we have described here in shaded fouling communities. Thus, microhabitat could play an important role in competitive outcomes under acidification, and shaded areas may provide a refuge from algal competition for those calcified filter feeders that are able to recruit under acidification, although see Celis-Plá *et al.* (2015) for examples of positive combined effects of shade and acidification on macroalgae.

The observed species-level changes at the acidified site, likely driven by direct effects and mediated by interspecific interactions, culminated in community-level shifts in structure. Our results conformed to the general expectation that communities experiencing high $p\text{CO}_2$ may shift from calcified to mainly non-calcified consumers (Christen *et al.*, 2013) likely due to energetic trade-offs which result in less energy available for calcification (Gaylord *et al.*, 2015). Our results compliment a growing number of studies documenting changes in community structure and diversity with increased $p\text{CO}_2$ in a range of habitat types (Kroeker *et al.*, 2013c; Campbell & Fourqurean, 2014; Meadows *et al.*, 2015; Raulf *et al.*, 2015; Sarmiento *et al.*, 2015; Brown *et al.*, 2016). The significant trend towards homogeneity among invertebrate communities under acidified conditions by the end of our experiment is similar to that described for fouling communities in western Canada (Brown *et al.*, 2016), and for algal communities close to CO_2 seeps (Porzio *et al.*, 2011; Kroeker *et al.*, 2013b).

Conclusions

We found that elevated $p\text{CO}_2$ conditions in the Mediterranean stimulated the initial colonization of settlement panels by biofilm. Despite the promotion of biofilm, succession was delayed at the acidified site and secondary colonization was lower. After eight weeks, however, subsequent succession accelerated quickly, resulting in higher secondary colonization, altered community structure, and a more homogeneous biofouling community than that found at ambient levels of $p\text{CO}_2$. Life history strategies, such as larval dispersal ability, environmental tolerances, growth rate and competitive ability, influence species-specific responses of organisms as they colonize new substrata, and are important to consider, even in closely related species (Gambi *et al.*, 2016). We found marked shifts in recruitment patterns which may alter routes of energy flow between trophic levels; later settlers may arrive out of sync with food sources, predators, or competitors (Dupont & Thorndyke, 2009; Nagelkerken & Connell, 2015). We also found that acidification altered community structure and these changes were driven both by past exposure (colonization history) and recent exposure to high $p\text{CO}_2$. Accelerated succession, homogenization, and changes to diversity under acidification occurred independently of colonization history. These processes might be driven more by proximate environmental conditions and small-scale within-site recruitment. The observed community-level shifts are therefore likely a result of not only persistent and transient effects of interspecific variability in response to increased $p\text{CO}_2$ but also, importantly, shifting interactions between and within primary and secondary colonizer guilds (Connell & Slatyer, 1977; Gaylord *et al.*, 2015). Overall, these short and longer-term acidification-driven changes in community succession could have important implications for ecosystem function and food web dynamics.

562

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References

- Arnold T, Mealey C, Leahey H, Miller A, Hall-Spencer JM, Milazzo M, Maers K (2012) Ocean acidification and the loss of phenolic substances in marine plants. *PLoS ONE*, **7**, e35107.
- Baggini C, Salomidi M, Voutsinas E, Bray L, Krasakopoulou E, Hall-Spencer JM (2014) Seasonality affects macroalgal community response to increases in $p\text{CO}_2$. *PLoS ONE*, **9**, e106520.
- Baggini C, Issaris Y, Salomidi M, Hall-Spencer J (2015) Herbivore diversity improves benthic community resilience to ocean acidification. *Journal of Experimental Marine Biology and Ecology*, **469**, 98–104.
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.
- Boatta F, D'Alessandro W, Gagliano AL et al. (2013) Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. *Marine Pollution Bulletin*, **73**, 485–494.
- Boyd PW, Cornwall CE, Davison A et al. (2016) Biological responses to environmental heterogeneity under future ocean conditions. *Global Change Biology*, 1–18.
- Brown NEM, Therriault TW, Harley CDG (2016) Field-based experimental acidification alters fouling community structure and reduces diversity. *Journal of Animal Ecology*, **85**, 1328–1339.
- Calosi P, Rastrick SPS, Lombardi C et al. (2013a) Adaptation and acclimatization to ocean acidification in marine ectotherms: an in situ transplant experiment with polychaetes at a shallow CO_2 vent system. *Proceedings of the Royal Society B: Biological Sciences*, **368**, 1–15.
- Calosi P, Rastrick SPS, Graziano M et al. (2013b) Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid–base and ion-regulatory abilities. *Marine Pollution Bulletin*, **73**, 470–484.
- Campbell JE, Fourqurean JW (2014) Ocean acidification outweighs nutrient effects in structuring seagrass epiphyte communities. *Journal of Ecology*, **102**, 730–737.
- Celis-Plá PSM, Hall-Spencer JM, Horta PA, Milazzo M, Korbee N, Cornwall CE, Figueroa FL (2015) Macroalgal responses to ocean acidification depend on nutrient and light levels. *Frontiers in Marine Science*, **2**.
- Chan F, Barth JA, Blanchette CA et al. (2017) Persistent spatial structuring of coastal ocean acidification in the California Current System. *Scientific Reports*, **7**, 2526.
- Christen N, Calosi P, McNeill C, Widdicombe S (2013) Structural and functional vulnerability to elevated $p\text{CO}_2$ in marine benthic communities. *Marine Biology*, **160**, 2113–2128.
- Cifuentes M, Krueger I, Dumont CP, Lenz M, Thiel M (2010) Does primary colonization or community structure determine the succession of fouling communities? *Journal of Experimental Marine Biology and Ecology*, **395**, 10–20.
- Cigliano M, Gambi M-C, Rodolfo-Metalpa R, Patti FP, Hall-Spencer JM (2010) Effects of ocean

acidification on invertebrate settlement at volcanic CO₂ vents. *Marine Biology*, **157**, 2489–2502.

Clark H, Gobler C (2016) Do diurnal fluctuations in CO₂ and dissolved oxygen concentrations provide a refuge from hypoxia and acidification for early life stage bivalves? *Marine Ecology Progress Series*, **558**, 1–14.

Connell JH, Slatyer R (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Society of Naturalists*, **111**, 1119–1144.

Connell SD, Kroeker KJ, Fabricius KE, Kline DI, Russell BD (2013) The other ocean acidification problem: CO₂ as a resource among competitors for ecosystem dominance. *Proceedings of the Royal Society B: Biological Sciences*, **368**, 1–9.

Cornwall CE, Revill AT, Hall-Spencer JM, Milazzo M, Raven JA, Hurd CL (2017) Inorganic carbon physiology underpins macroalgal responses to elevated CO₂. *Scientific Reports*, **7**, 46297.

Donnarumma L, Lombardi C, Cocito S, Gambi MC (2014) Settlement pattern of *Posidonia oceanica* epibionts along a gradient of ocean acidification : an approach with mimics. *Mediterranean Marine Science*, **15**, 498–509.

Doropoulos C, Diaz-Pulido G (2013) High CO₂ reduces the settlement of a spawning coral on three common species of crustose coralline algae. *Marine Ecology Progress Series*, **475**, 93–99.

Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby P (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. *Ecology Letters*, **15**, 338–346.

Dupont S, Thorndyke MC (2009) Impact of CO₂-driven ocean acidification on invertebrates early life-history – what we know, what we need to know and what we can do. *Biogeosciences Discussions*, **6**, 3109–3131.

Fabricius KE, Langdon C, Uthicke S et al. (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate Change*, **1**, 165–169.

Fabricius KE, De’ath G, Noonan S, Uthicke S (2014) Ecological effects of ocean acidification and habitat complexity on reef-associated macroinvertebrate communities. *Proceedings of the Royal Society B: Biological Sciences*, **281**.

Feely RA, Sabine CL, Lee K et al. (2004) Impact of anthropogenic CO₂ on the CaCO₃ system in the oceans. *Science*, **305**, 362–366.

Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive “acidified” water onto the continental shelf. *Science*, **320**, 1490–1492.

Fortunato H (2015) Bryozoans in climate and ocean acidification research: A reappraisal of an under-used tool. *Regional Studies in Marine Science*, **2070**.

Fournier DA, Skaug HJ, Ancheta J et al. (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, **27**, 233–249.

Gambi MC, Musco L, Giangrande A, Badalamenti F, Micheli F, Kroeker KJ (2016) Distribution

and functional traits of polychaetes in a CO₂ vent system: Winners and losers among closely related species. *Marine Ecology Progress Series*, **550**, 121–134.

Garilli V, Rodolfo-Metalpa R, Scuderi D et al. (2015) Physiological advantages of dwarfing in surviving extinctions in high-CO₂ oceans. *Nature Climate Change*, **5**, 1–6.

Gaylord B, Kroeker KJ, Sunday JM et al. (2015) Ocean acidification through the lens of ecological theory. *Ecology*, **96**, 3–15.

Goodwin C, Rodolfo-Metalpa R, Picton B, Hall-Spencer JM (2014) Effects of ocean acidification on sponge communities. *Marine Ecology*, **35**, 41–49.

Gounand I, Kefi S, Mouquet N, Gravel D (2016) Trait selection during food web assembly: the roles of interactions and temperature. *Theoretical Ecology*, **9**, 417–429.

Guadayol Ò, Peters F, Marrasé C et al. (2009) Episodic meteorological and nutrient-load events as drivers of coastal planktonic ecosystem dynamics: A time-series analysis. *Marine Ecology Progress Series*, **381**, 139–155.

Hadfield MG (2011) Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science*, **3**, 453–470.

Hale R, Calosi P, McNeill L, Mieszkowska N, Widdicombe S (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos*, **120**, 661–674.

Hall-Spencer JM, Allen R (2015) The impact of CO₂ emissions on “ nuisance ” marine species. *Research and Reports in Biodiversity Studies*, **4**, 33–46.

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S et al. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, **454**, 96–99.

Hallett LM, Jones SK, MacDonald AAM et al. (2016) codyn: An r package of community dynamics metrics. *Methods in Ecology and Evolution*, **7**, 1146–1151.

Harvey BP, McKeown NJ, Rastrick SPS et al. (2016) Individual and population-level responses to ocean acidification. *Scientific Reports*, **6**, 20194.

Henson SA, Beaulieu C, Ilyina T et al. (2017) Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nature Communications*, **8**, 14682.

Hofmann GE, Smith JE, Johnson KS et al. (2011) High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE*, **6**, e28983.

Hönisch B, Ridgwell A, Schmidt DN et al. (2012) The geological record of ocean acidification. *Science*, **335**, 1058–63.

Iles AC, Gouhier TC, Menge BA, Stewart JS, Haupt AJ, Lynch MC (2012) Climate-driven trends and ecological implications of event-scale upwelling in the California Current System. *Global Change Biology*, **18**, 783–796.

Johnson V, Russell B, Fabricius KE, Brownlee C, Hall-Spencer JM (2012) Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO₂ gradients. *Global Change Biology*, **18**, 2792–2803.

Johnson R, Brownlee C, Rickaby R, Graziano M, Milazzo M, Hall-Spencer JM (2013)

Responses of marine benthic microalgae to elevated CO₂. *Marine Biology*, **160**, 1813–1824.

Johnson V, Brownlee C, Milazzo M, Hall-Spencer J (2015) Marine microphytobenthic assemblage shift along a natural shallow-water CO₂ gradient subjected to multiple environmental stressors. *Journal of Marine Science and Engineering*, **3**, 1425–1447.

Kroeker KJ, Micheli F, Gambi M-C, Martz TR (2011) Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proceedings of the National Academy of Sciences*, **108**, 14515–14520.

Kroeker KJ, Kordas RL, Crim R et al. (2013a) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*, **19**, 1884–1896.

Kroeker KJ, Micheli F, Gambi M-C (2013b) Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change*, **3**, 156–159.

Kroeker KJ, Gambi M-C, Micheli F (2013c) Community dynamics and ecosystem simplification in a high-CO₂ ocean. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 12721–6.

Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO₂ on early development in the mussel *Mytilus galloprovincialis*. *Aquatic Biology*, **4**, 225–233.

Lancaster L, Morrison G, Fitt R (2016) Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. *Philosophical Transactions of the Royal Society B*.

Lane AC, Mukherjee J, Chan VBS, Thiyagarajan V (2013) Decreased pH does not alter metamorphosis but compromises juvenile calcification of the tube worm *Hydroides elegans*. *Marine Biology*, **160**, 1983–1993.

Li C, Chan V, He C, Meng Y (2014) Weakening mechanisms of the serpulid tube in a high CO₂ world. *Environmental Science & Technology*, **48**, 14158–14167.

Li F, Wu Y, Hutchins DA, Fu F, Gao K (2016) Physiological responses of coastal and oceanic diatoms to diurnal fluctuations in seawater carbonate chemistry under two CO₂ concentrations. *Biogeosciences*, **13**, 6247–6259.

Lidbury I, Johnson V, Hall-Spencer JM, Munn C, Cunliffe M (2012) Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. *Marine Pollution Bulletin*, **64**, 1063–1066.

Linares C, Vidal M, Canals M et al. (2015) Persistent natural acidification drives major distribution shifts in marine benthic ecosystems. *Proceedings of the Royal Society B*, **282**, e20150587.

Meadows AS, Ingels J, Widdicombe S, Hale R, Rundle SD (2015) Effects of elevated CO₂ and temperature on an intertidal meiobenthic community. *Journal of Experimental Marine Biology and Ecology*, **469**, 44–56.

Milazzo M, Rodolfo-Metalpa R, Chan VBS et al. (2014) Ocean acidification impairs vermetid reef recruitment. *Scientific Reports*, **4**, 1–7.

Milazzo M, Cattano C, Alonzo SH et al. (2016) Ocean acidification affects fish spawning but not

732 paternity at CO₂ seeps. *Proceedings of the Royal Society B*, **283**, 20161021.

733 Nagelkerken I, Connell SD (2015) Global alteration of ocean ecosystem functioning due to
 734 increasing human CO₂ emissions. *Proceedings of the National Academy of Sciences*, **2015**,
 735 201510856.

736 Oksanen AJ, Blanchet FG, Kindt R et al. (2015) vegan: Community Ecology package.

737 Osman R, Whitlatch R, Zajac R (1989) Effects of resident species on recruitment into a
 738 community larval settlement versus post-settlement mortality in the oyster *Crassostrea*
 739 *virginica*. *Marine Ecology Progress Series*, **54**, 61–73.

740 Peck LS, Clark MS, Power D, Reis J, Batista FM, Harper EM (2015) Acidification effects on
 741 biofouling communities: winners and losers. *Global Change Biology*, **21**, 1907–1913.

742 Pickett M, Andersson AJ (2015) Dissolution rates of biogenic carbonates in natural seawater at
 743 different pCO₂ conditions: a laboratory study. *Aquatic Geochemistry*, 459–485.

744 Platt WJ, Connell JH (2003) Natural disturbances and directional replacement of species.
 745 *Ecological Monographs*, **73**, 507–522.

746 Porzio L, Buia MC, Hall-spencer JM, Cristina M (2011) Effects of ocean acidification on
 747 macroalgal communities. *Journal of Experimental Marine Biology and Ecology*, **400**, 278–
 748 287.

749 Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple
 750 abiotic stressors on marine embryos and larvae. *Global change biology*, **21**, 2122–2140.

751 Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal
 752 waters. *Ices Journal of Marine Science*, **66**, 1528–1537.

753 R Development Core Team (2009) R: a language and environment for statistical computing.

754 Raulf FF, Fabricius K, Uthicke S, de Beer D, Abed RMM, Ramette A (2015) Changes in
 755 microbial communities in coastal sediments along natural CO₂ gradients at a volcanic vent
 756 in Papua New Guinea. *Environmental Microbiology*, **17**, 3678–3691.

757 Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO₂-
 758 induced ocean acidification. *Geology*, **37**, 1131–1134.

759 Ross P, Parker L, O'Connor W, Bailey EA (2011) The Impact of ocean acidification on
 760 reproduction, early development and settlement of marine organisms. *Water*, **3**, 1005–1030.

761 Ross PM, Parker L, Byrne M (2016) Transgenerational responses of molluscs and echinoderms
 762 to changing ocean conditions. *ICES Journal of Marine Science*, **73**, 537–549.

763 Russell BD, Connell SD, Findlay HS, Tait K, Widdicombe S, Mieszkowska N (2013) Ocean
 764 acidification and rising temperatures may increase biofilm primary productivity but
 765 decrease grazer consumption. *Proceedings of the National Academy of Sciences of the*
 766 *United States of America*, **368**.

767 Sams MA, Keough MJ (2012) Contrasting effects of variable species recruitment on marine
 768 sessile communities. *Ecology*, **93**, 1153–1163.

769 Sanford E, Gaylord B, Hettinger A, Lenz EA, Meyer K, Hill TM (2014) Ocean acidification
 770 increases the vulnerability of native oysters to predation by invasive snails. *Proceedings of*

771 *the Royal Society B: Biological Sciences*, **281**, 1–8.

772 Sarmiento VC, Souza TP, Esteves a. M, Santos PJP (2015) Effects of seawater acidification on a
773 coral reef meiofauna community. *Coral Reefs*, **34**, 955–966.

774 Schneider CA, Rasb WS, Eliceiri KW, Rasband WS (2012) NIH Image to ImageJ: 25 years of
775 image analysis. *Nature Methods*, **9**, 671–675.

776 Shaw E, Munday P, McNeil B (2013) The role of CO₂ variability and exposure time for
777 biological impacts of ocean acidification. *Geophysical Research Letters*, **40**, 4685–4688.

778 Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker B (2013) glmmADMB: generalized
779 linear mixed models using AD model builder. R package version 0.7.7.4.

780 Small DP, Milazzo M, Bertolini C et al. (2015) Temporal fluctuations in seawater pCO₂ may be
781 as important as mean differences when determining physiological sensitivity in natural
782 systems Daniel. *Ices Journal of Marine Science*, **73**, 1–9.

783 Smith AM, Garden CJ (2013) Being a bimineralic bryozoan in an acidifying ocean. , Vol. 143
784 (eds Ernst A, Schafer P, Scholz J), pp. 137–153. Berlin, Heidelberg.

785 Smith AM, Key MM, Gordon DP (2006) Skeletal mineralogy of bryozoans: Taxonomic and
786 temporal patterns. *Earth-Science Reviews*, **78**, 287–306.

787 Smith AM, Riedi MA, Winter DJ (2013) Temperate reefs in a changing ocean: Skeletal
788 carbonate mineralogy of serpulids. *Marine Biology*, **160**, 2281–2294.

789 Sousa WP (1979) Experimental investigations of disturbance and ecological succession in a
790 rocky intertidal algal community. *Ecological Monographs*, **49**, 227–254.

791 Sunday JM, Fabricius KE, Kroeker KJ et al. (2017) Ocean acidification can mediate biodiversity
792 shifts by changing biogenic habitat. *Nature Climate Change*, **7**, 81–85.

793 Sutherland JP (1974) Multiple stable points in natural communities. *American Naturalist*, **108**,
794 859–873.

795 Tans P (2009) An accounting of the observed increase in oceanic and atmospheric CO₂ and the
796 outlook for the future. *Oceanography*, **22**, 26–35.

797 Taylor PD, Lombardi C, Cocito S (2015) Biomineralization in bryozoans: present, past and
798 future. *Biological Reviews*, **90**, 1118–1150.

799 Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general
800 principles. *Ecology*, **80**, 1455–1474.

801 Vaz-Pinto F, Olabarria C, Gestoso I, Cacabelos E, Incera M, Arenas F (2013) Functional
802 diversity and climate change: effects on the invasibility of macroalgal assemblages.
803 *Biological Invasions*, **15**, 1833–1846.

804 Vizzini S, Di Leonardo R, Costa V, Tramati CDD, Luzzu F, Mazzola A (2013) Trace element
805 bias in the use of CO₂ vents as analogues for low pH environments: Implications for
806 contamination levels in acidified oceans. *Estuarine, Coastal and Shelf Science*, **134**, 19–30.

807 Webster N, Uthicke S, Botté E, Flores F, Negri A (2013) Ocean acidification reduces induction
808 of coral settlement by crustose coralline algae. *Global Change Biology*, **19**, 303–315.

809 Wittmann AC, Pörtner H-O (2013) Sensitivities of extant animal taxa to ocean acidification.
810 *Nature Climate Change*, **3**, 995–1001.

811 Zhan A, Briski E, Bock DG, Ghabooli S, Macisaac HJ (2015) Ascidiaceans as models for studying
812 invasion success. *Marine Biology*, **162**, 2449–2470.

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Table legends

Table 1. Carbonate chemistry of source seawater from ambient and high CO₂ sites. Temperature, salinity, pH_{NBS}, and total alkalinity were collected every two weeks from March to June 2013 (mean +/- SE, n =). Asterisks indicate calculated values in the CO₂-SYS program (Pierrot et al. 2006).

Table 2. Results of GLMMs (z statistic) and LME models (using X^2) using percent cover of a given species and site as fixed effects. Week 2-8 n=20, week 12 n=10. ^p indicates week in which peak % cover of this species occurred.

Table 3. Results of GLMMs using percent cover of a given species and initial site, final site and their interaction as fixed effects (n=20).

Figure Legends

Fig. 1. Photographs depicting sites and pH gradient (left) and the panel and tile system. Two panels (centre), with 10 PVC tiles (right) attached to the underside of each panel, were suspended ~1m from both surface and bottom using a buoy and anchor system.

Fig. 2. Abundance of selected primary colonizers in ambient and low pH sites over time, left-hand panels are up to week 8 (n=20) and right-hand panels are at week 12 (n=10) of both transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis indicates final site. Species are: (a, b) biofilm (% cover), (c, d) *Cladophora* sp. (% cover), (e, f) serpulids (# individuals) and, (g, h) spirorbids (# individuals). Error bars indicate standard error.

Fig. 3. Abundance of selected secondary colonizers in ambient and low pH sites over time, left-hand panels are up to week 8 (n=20) and right-hand panels are at week 12 (n=10) of both transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis indicates final site. Species are: (a, b) *Diplosoma* sp. (% cover), (c, d) *Botryllus* sp. (% cover), (e, f) Thin ramified bryozoan (% cover) and, (g, h) *Schizomavella* sp. (# colonies). Error bars indicate standard error.

Fig. 4. Fig. 4. nMDS ordination plot showing the relationship between communities after (a) 8 weeks on tiles from low pH (open circles) vs. the ambient site (solid black triangles), n = 20 tiles and (b) 12 week on tiles that (1) remained in low pH (open circles), (2) were transplanted from low pH to the ambient site (solid blue circles), (3) were transplanted from ambient site to low pH site (red open triangles), and (4) remained in the ambient site (solid black triangles), n = 10 tiles.

Fig. 5. Community-wide measures in ambient and low pH sites over time, left-hand panels are up to week 8 (n=20) and right hand panels are at week 12 (n=10) of both transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis indicates final site. Measures are: (a, b) total occupied space, (c, d) secondary colonizers space

851 occupation, (e, f) Shannon's diversity, and (g, h) species richness. Error bars indicate standard
852 error.